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Screening of Some Herbal Medicine Preparations for Anti-Diabetic Activity

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Abstract:
Background: Management of Diabetes Mellitus (DM) has become a huge challenge in the present-day society owing to the increasing cost of medicine and the global economic meltdown. Seeking for cheaper, effective and natural alternatives is preferential as the world is now moving towards the use of medicinal plant therapy. There is need for authentication of already-existing herbal preparations for DM in order to ensure safety and efficacy. This study therefore aimed to experimentally evaluate the hypoglycemic activity of some acclaimed Anti-Diabetic Herbal Preparations (acADHP) used in parts of North-Central, Nigeria for herbal treatment of DM.
Materials and Methods: Fifty healthy young male albino mice (Wistar strain) weighing between 20-30g were divided into ten groups. Animals were made diabetic by administration of 50mg/kg body wt. streptozotocin followed three days later by the anti-diabetic preparation collected from the various Herbal Medicine Practitioners. Blood glucose concentration (BGC) of the animals were determined on days 5, 7, 9, 11, 13 and 15. These BCGs were compared with controls.
Results: The acADHP labeled BSA/1, JJN/2, BLD/1, LFA/2 and AKW/3 possessed significant ($p<0.05$)hypoglycemic activity. Herbal preparations JJN/2 and LFA/2 elicited hypoglycemic activity that was close to that of tolbutamide. Furthermore, it was observed that all the herbal preparations were made from V. amygdalina and P. americana.
Conclusion: Commonly used herbal preparations for the management of Diabetes Mellitus (DM) by Herbal Medicine Practitioners (HMPrs) have hypoglycemic activity as it lowered the blood glucose concentration levels of Streptozotocin-induced hyperglycemic mice.
Key Word: Herbal medicines; Diabetes mellitus; P. americana; V. amygdalina; Hypoglycemic activity; North-central, Nigeria.

I. Introduction

Herbal medicine practice is referred to as alternative medicine (AM) from the orthodox medicine (OM) perspective as it does not strictly adhere to scientific approach [1], thus attracted little attention over the years.Recently, same "alternative medicine" and "natural therapies" have attracted interests of researchers as they are widely prescribed a result of perceived effectiveness, minimal side effects in clinical experience and relatively low costs despite their unknown bioactive constituents [2]. Modern medicine today utilizes bioactive compounds isolated from higher plants, and about 80% of the isolated active ingredients indicate positive correlation between modern therapeutic and traditional uses [3]. Over 60% of the world's population and about 80% in developing countries depend on medicinal plants for their medical care [4]. This points to the fact that the entire world recognizes and engage in the use of traditional medicine. The prevalence of diabetes mellitus (DM) is increasing worldwide and it is projected that by the year 2030 over 500millions adult will be affected by the disease [5]. According to the WHO predictions, DM will become the seventh leading cause of death in the world by the year 2030 [6]. Furthermore, the WHO estimated a 4.3% prevalence of diabetes in Nigeria in 2016 [7]. Previous studies also asserted that, about 4.7 million Nigerians had type 2 diabetes [8] with a prevalence of between 0.8% and 11% [9].

Diabetes is a chronic metabolic disorder and many plant-based materials are used in traditional medicine practice to treat this [10]. The disorder poses a huge health burden. In the long term, effects can lead to among others micro and macro vascular abnormalities [11]. Diabetes mellitus is a syndrome characterized by irregular production and release and/or action of insulin which subsequently disrupts glucose metabdiem[12]. People with diabetes are more prone to developing different life-threatening health problems which attracts more unplanned expenditure for medical care, thus lowering the life quality and increasing mortality rate [13]. Type 2-Diabetes mellitus (T2DM) also called maturity/ (adult-onset DM is caused by decrease responsiveness of tissues to the effects of insulin or diminished production and release of insulin from the β-cells of the islets of Langerhans, while Type 1DM or Juvenile onset DM is an autoimmune disorder where the β-cells are completely destroyed by autoimmune antibodies [14]. Diabetic patients also exhibit "oxidative stress", which leads to lipid peroxidation and tissue damage including retinopathy, nephropathy, neuropathy and coronary heart disease[15]. According to statistics, 2.8% of the world's population suffer from this disease and it is expected to increase to more than 5.4% by 2025 [16]. The number of people with diabetes today has been growing and causing increasing concerns in medical community and the public [10] at large.

Many medicinal plants have been known for their hypoglycemic/antidiabetic properties following scientific evaluation. This includes the *Aloe barbadensis Miller* plant (*Aloe vera*) used in traditional medicine by many people. Treatment with the ethanolic extract of the fresh leaf gel (300 and 500 mg/kg) in STZ-induced hyperglycemic rats for 42 days resulted in a significant decrease in the fasting blood glucose levels [17]. *Phyllanthus amarus* - leaves known as a hypoglycemic factor in central and southern India. Oral administration of ethanolic extract from (400 mg/kg/BW) for 45 days caused a significant reduction in blood glucose concentration in alloxan-induced hyperglycemic mice [18], Laboratory studies reveal that oral administration of ethanolic extract of *Bryonia alba* root (200mg/kg) for 7 days cause a significant decrease in blood glucose concentration in alloxan-induced hyperglycemic rats with confirmation of the historic claim of the anti-diabetic property of the plant [19]. Furthermore, the chloroform extract of *Acacia arabica* bark was used for 2 weeks in diabetic rats to significantly reduce the blood glucose concentration and improved the cholesterol, triglyceride, HDL, and LDL levels [20].

High cost of management of DM especially in cases of complications in low income earning countries e.g., Nigeria where National Health Insurance Scheme (NHIS) coverage is < 5% in which the insurance coverage benefits the high working class [21] are palpable indicators to refer to DM as an "expensive disease". An effective, affordable, safer and reachable natural product alternative becomes imperative in order to tackle the palpable challenges surrounding the management of DM in the 3rd world countries. This study aimed to experimentally evaluate the hypoglycemic activity of some acclaimed Anti-Diabetic Herbal Preparations (acADHP) used in parts of North-Central, Nigeria.

II. Material And Methods

The preliminary study involved a survey of herbal medicine practitioners (HMPrs) cutting across a total of 8 Local Government Areas (LGA), made up of 5 LGAs in Plateau and 3 LGAs in Nasarawa states of North-Central, Nigeria. Their protocols for the management of DM were thoroughly scrutinized and documented.
Experimental laboratory report on hyperglycemic
Sample Collection: Already-prepared herbal medicines for the treatment of DM were collected from 23 HMPrs in Bassa, Jos North, Jos South, BarkinLadi and Pankshin LGAs of Plateau State and Lafia, Akwanga and Keffi LGAs of Nasarawa State of Nigeria.
Eight out of the 23 HMPrs gave samples of their herbal preparations were identified and recruited for the study which were stored in tightly closed bottles maintained at 3-4^{0}C storage temperature for subsequent evaluation and pharmacological screening for hypoglycemic activity.

The HMPrs revealed the composition of their herbal preparations as follows:

- JJN/2, LFA/2 and AKW/3 were prepared from the stem bark of *P. americana*.
- BSA/1 and BLD/1 were prepared from the leaves of *V. amygdalina*.
- BUU/3 and KEF/1 were prepared from seeds of *P. americana* +2-3g of potash.
- PKN/3 was prepared from the leaves and branches of *V. amygdalina* +2-3g of potash.

The methods of preparation by different herbal medicine practitioners were by boiling in water and using the supernatant on cooling for the treatment of DM.

Geographically, Plateau State is located in the North Central Zone out of the six geopolitical zones of Nigeria.[22], It is the twelfth-largest state in Nigeria, approximately in the centre of the country, and geographically unique in Nigeria due to its boundaries of elevated hills surrounding the Jos Plateau [23] . Plateau State is described as "The Home of Peace and Tourism". With natural formations of rocks, hills and waterfalls, it derives its name from the Jos Plateau and has a population of around 3.5 million people [24].

Nasarawa State is a state in the North Central region of Nigeria, bordered to the east by the states of Taraba and Plateau, to the north by Kaduna State, to the south by the states of Kogi and Benue, and to the west by the Federal Capital Territory. Named for the historic Nasarawa Emirate, the state was formed from the west of Plateau State on 1 October 1996. [25]. Of the 36 states of Nigeria, Nasarawa is the fifteenth largest in area and second least populous with an estimated population of about 2.5 million as of 2016. [26]. Geographically, the state is mostly within the tropicalGuinean forest–savanna mosaicecoregion. Important geographic features include the River Benue forming much of Nasarawa State's southern borders and the state's far northeast containing a small part of the Jos Plateau[27].

Table no 1: Maps showing various location of herbal sample collection

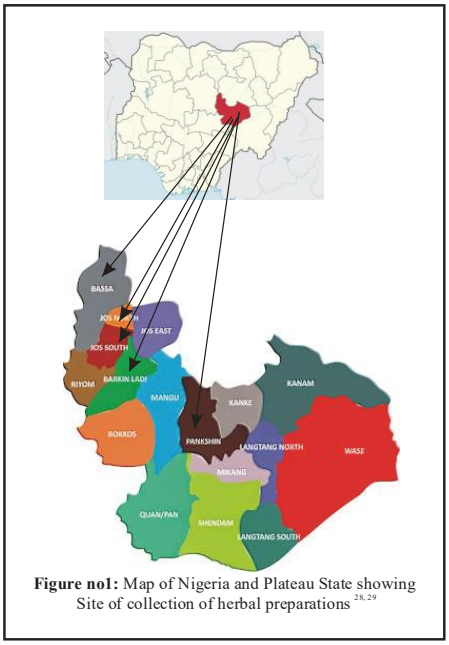

Figure no1: Map of Nigeria and Plateau State showing Site of collection of herbal preparations [28, 29]

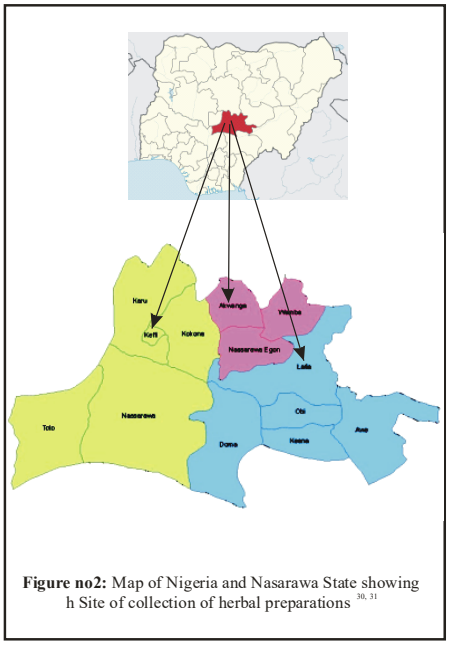

Figure no2: Map of Nigeria and Nasarawa State showing h Site of collection of herbal preparations [30, 31]

Drugs

- Streptozotocin (STZ) and α-d-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA). This was used in 0.1 M sodium citrate buffer, pH 4.5.
- Tolbutamide tablet - Mylan Pharmaceuticals Inc (Canonsburg, Pennsylvania, USA).

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Procurement, Preparation of Experimental Animals and Protocols: Fifty young healthy male albino mice – Wistar strain (previously made hyperglycemic by administration of 50mg/kg IP STZ) were divided into 10 groups (labeled I-X). Animals were allowed water (distilled water) *ad libitum*. BGC of each animal was measured using a glucose meter (One Touch Ultra glucometer-Accu-Check Active, Roche) in the morning of days 1 and 3 after an overnight food fast (to confirm sustained hyperglycemic status). On the morning of day 5, the drinking water of animals in groups II to X was replaced by the herbal preparations as outlined in table below, while animals in group were given water containing tolbutamide 1mg/ml. animals in group I continued to drink distilled water.

The animals in the 10 groups were identified with group number (I -X), site of collection of already-prepared herbal medicines used for the treatment of diabetes administered and identification code number of the herbal preparation as below:

Group I (Control) – Distilled water (DW)
Group II – Herbal medicine collected from Bassa LGA, Plateau state, Nigeria (BSA/1).
Group III – Herbal medicine collected from Jos North LGA, Plateau state, Nigeria (JJN/2).
Group IV – Herbal medicine collected from Jos South LGA, Plateau state, Nigeria (BUU/3).
Group V – Herbal medicine collected from BarkinLadi LGA, Plateau state, Nigeria (BLD/1).
Group VI – Herbal medicine collected from Pankshin LGA, Plateau state, Nigeria (PKN/3).
Group VII – Herbal medicine collected from Lafia LGA, Nasarawa state, Nigeria (LFA/2).
Group VIII – Herbal medicine collected from Akwanga LGA, Nasarawa state, Nigeria (AKW/3).
Group IX – Herbal medicine collected from Keffi LGA, Nasarawa state, Nigeria (KEF/1).
Group X – 1mg/ml tolbutamide (TBD) i.e reference drug.
The experiment was carried for a period of 15 days.

Blood Glucose Concentration (BGC) of each animal was subsequently determined using a glucometer (Accu-Check Active, Roche) on days 5, 7, 9, 11, 13 and 15. Moreover, BGC was plotted against time (days) for each group, from which the effect of each herbal preparation was compared with that of control and tolbutamide (standard drug). Subsequent information from interactions with the HMPs from whom the herbal medicines were collected were also noted.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) and graphs. The level $P < 0.05$ was considered as the cutoff value or significance. The SPSS version 20 (SPSS Inc., Chicago, IL) statistical package was used for analysis.

III. Result

The herbal preparations labeled BSA/1, JJN/2, BLD/1, LFA/2 and AKW/3 administered in place of drinking water to animals in groups II, III, V, VII and VIII respectively, possessed significant ($p<0.05$)hypoglycemic activity (Table no1, Figures no 2 and 3).

Table no1: Blood glucose concentration values following liberal ingestion of the different herbal preparations

Group	Sample	Blood glucose concentration (Mean +SEM) mg/dL					
		Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
I	DW	145±3.9	148±5.2	153±3.5	148±5.3	157±4.5	157±4.5
II	BSA/1	143±3.9	122±4.2*	106±4.8*	111±4.7*	104±3.6*	112±4.5
III	JJN/2	159±4.0	94±3.7*	84±2.9*	78±3.9*	83±4.8*	82±3.6*
IV	BUU/3	155±3.2	152±4.9	146±5.4	159±3.2	151±3.3	150±4.1
V	BLD/1	140±4.8	108±2.9*	115±4.7*	117±5.8	114±4.2*	118±3.4*
VI	PKN/3	154±6.1	143±3.6	149±5.4	158±4.8	159±4.8	162±6.3
VII	LFA/2	148±4.8	103±5.4*	91±4.1*	98±4.2*	89±3.2*	82±3.7*
VIII	AKW	142±5.6	119±4.1	126±4.1*	112±5.1*	102±4.0*	98±4.4
IX	KEF/1	150±2.8	153±3.6	145±5.3	145±3.4	149±3.6	153±6.2
X	Tolbutamide	138±3.4	87±4.5*	76±3.6*	85±4.1*	75±3.2*	67±3.3*

** $p<0.05$ versus corresponding control, $n = 5$*

Table no1 Shows BGC (mg/dL) values for days 5, 7, 9, 11, 13 and 15 following liberal ingestion of the different herbal preparations collected from the selected HMPrs from Plateau and Nasarawa states. Generally, there was decrease in the BCG levels of the STZ-induced hyperglycemic mice following an initial slightincrease in BGC levels across the group at day 7 compared with day 5, except samples KEF /1 (153±3.6) which is close to sample DW (Negative control).

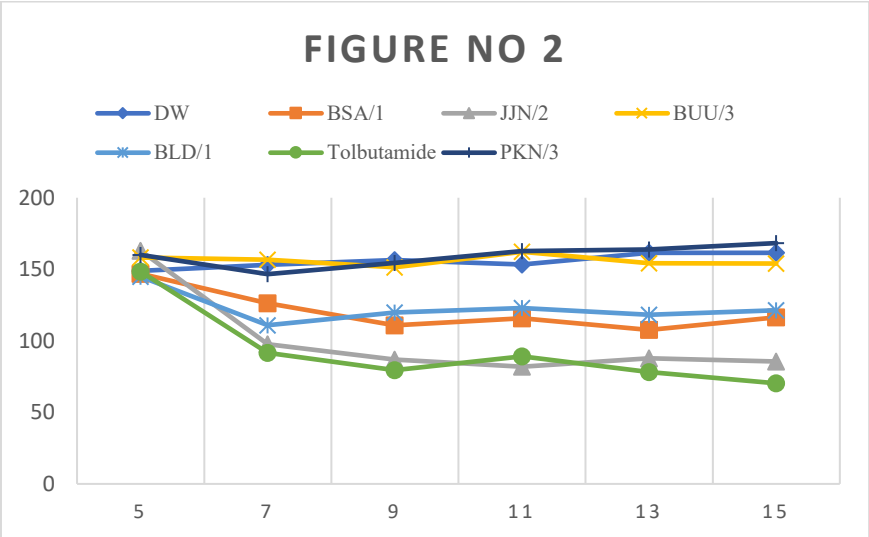

Figure no2: BGC values of mice following liberal ingestion of the acclaimed anti-diabetic herbal preparations (acADHP) collected from selected HMPrs in Plateau State

The hypoglycemic effect of the acADHP administered to the mice followed same pattern with tolbutamide administered group across the 15 days. Hypoglycemic effect was highest with sample JJN/2, followed by BSA/1, BLD/1 and BUU/3 respectively. Sample PKN/3 administered group had the least (transient) hypoglycemic effect compared to DW (Negative control). Samples JJN/2, BSA/1 and BLD/1 possessed significant ($p<0.05$)hypoglycemic activity – *Figure no2*.

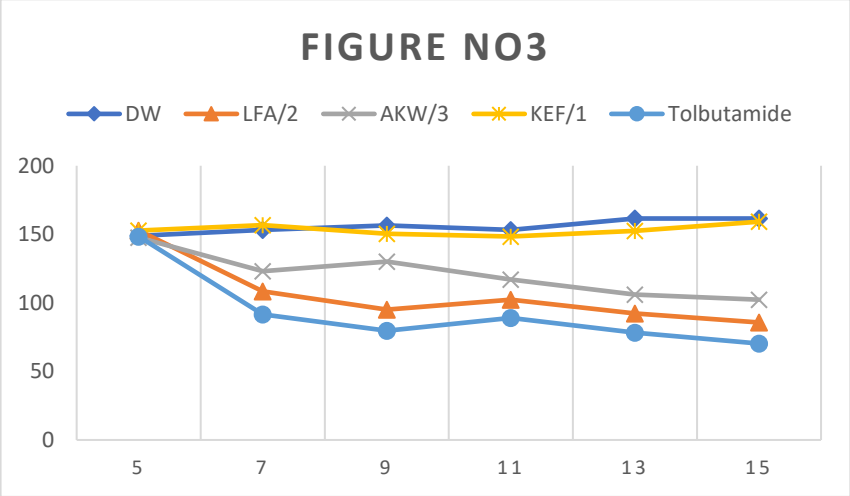

Figure no3: BGC values of mice following liberal ingestion of the different herbal preparations collected from selected HMPrs in Nasarawa State

Hypoglycemic effect of the AcADHP from Nasarawa state, administered to the mice also followed same pattern with tolbutamide administered group across the 15 days. The effect was highest with sample LFA/2, followed by AKW/3 and KEF/1 respectively. Samples LFA/2 and AKW/3 possessed significant ($p<0.05$)hypoglycemic activity – *Figure no3*.

IV. Discussion

In this present study, DM status of mice was achieved via intraperitoneal administration of STZ. Streptozotocin causes the destruction of β-cells of islets of Langerhans[32]33resulting in hyperglycemia which resemble human DM. Elevation of BGC levels that is characterized by decrease in insulin level in the STZ-induced diabetic mice were lowered by the various orally administered acADHPs. This hypoglycemic effect by same orally administered acADHPs might be by way of increased secretion of insulin from the remaining β–cells via potentiation of insulin or by increasing peripheral glucose uptake [33].

Sequel to the information via interactions with the HMPrs from whom the herbal medicines were collected, all the preparation i.e., *V. amygdalina* and *P. americana*irrespective of the methods of preparation (boiling in water and using the supernatant on cooling). Sani *et al*., 2019 reported that combination of different plants or their parts in the preparation of the recipes for the treatment of diabetes was common among a group of sampled herbal medicine practitioners[34]. It is evident that *V. amygdalina* and *P. americana* are common plant species used across the Plateau and Nasarawa States in North central-Nigeria following the inter-cultural relationship as both states fall within same vegetation belt and common rich plant diversity. The foregoing is consonance with Caballero-Serrano *et al*., 2019 who reported that the local usage of medicinal plants is related to plant diversity and traditional knowledge[35]. Furthermore, increase in patronage of the herbal products as pointed by the HMPrsin our study could be said to be in tandem with Rupeshkumar*et al.,* 2014 who reported that there is an increasing demand for the use of natural products as a result of side effects associated with insulin and oral hypoglycemic agents [36].

From the present study, hypoglycemic effect of the herbal preparations in the hyperglycemic mice was similar to that of Tolbutamide, which is a relatively moderate acting anti-diabetic agent - Fig*ure no2* and Fig*ure no3*. Two of the administered acADHPs shows hypoglycemic activity close to Tolbutamide as well, suggesting a seeminglyrelated mechanism of action.

This study also shows that, the hypoglycemic effect of the administered acADHPs made up of *V. amygdalina* and *P. americana* may have been due to the presence of phytoconstituents, including alkaloids, flavonoids, polyphenols, saponins and tannins that are known for their antidiabetic effect [37, 33, 38].

V. Conclusion

Commonly used herbal preparations for the management of Diabetes Mellitus (DM) used by Herbal Medical Practitioners (HMPrs) cutting across Plateau and Nasarawa States of North Central Nigeria lowered the blood glucose concentration levels of Streptozotocin-induced diabetic mice. Furthermore, all the sampled herbal preparations contain *V. amygdalina* and *P. americana*. The results support the use of these herbal remedies for the treatment of DM.

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